## 60 Evaluation of twice-a-year antibiotic susceptibility testing for *Pseudomonas aeruginosa* in adult patients with cystic fibrosis

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**Objectives:** In cystic fibrosis (CF), discrepancies have been reported between antibiotic susceptibility for *Pseudomonas aeruginosa* (Pa) and clinical efficacy. Given the "hands-on" time needed for the agar diffusion method that is used for Pa antibiotic susceptibility testing (AST), stringent cost constraints prompted us to reconsider our politics of systematically assaying all Pa isolates obtained from sputum samples of CF patients.
**Methods:** In a cohort of adult CF patients, we have reduced the frequency to which AST was performed on Pa isolates to not more than once every six months: when AST was performed less than six months before, only the presence and quantification of Pa was mentioned to the clinician. This change was monitored over a 1-year period. All isolates (with or without AST) were kept frozen and the clinician had the possibility to selectively request for unscheduled AST if needed.
**Results:** 1578 CF sputum samples from 444 CF patients were sent to the laboratory over one year (3.5 sample/patient). 844 samples (54%) were positive with $>10^3$ CFU/mL Pa. AST of Pa was done for 386 samples (46% of Pa-positive samples). 250 patients (56%) had at least one sample positive with Pa. The average annual number of AST was 1.5 per Pa-positive patient, 95% of them having at least one AST over one year. The clinicians requested extra AST in only one case over the analysed time period.
**Conclusion:** Reducing by approximately one half the frequency of AST for Pa isolates did not lead clinicians to ask for additional AST, and the costs related to Pa AST were significantly reduced. These results might be of interest for reevaluating the frequency of Pa AST in adult CF patients.

## 61 Baseline data from a prospective 5-year registry study to monitor susceptibility to aztreonam of *Pseudomonas aeruginosa* (*PA*) isolates from patients with cystic fibrosis (CF) in the United States

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**Objectives:** A Phase 4 observational study is prospectively monitoring *PA* susceptibility patterns and clinical data in a subset of subjects in the CF Foundation (CFF) Registry over a 5-yr period after introduction of Cayston® (AZLI).
**Methods:** Subjects ⩾6 yrs old, $FEV_1$ 25% to 90% predicted and documented ⩾2 *PA*-positive respiratory tract cultures were recruited from CFF-accredited centers. Sputum samples are collected at baseline and once yearly for 5 yrs and processed at a central reference laboratory. Study evaluations include *PA* susceptibility to aztreonam and other antibiotics; relationship between number of AZLI treatment courses and *PA* resistance to aztreonam (parenteral susceptibility breakpoint: MIC >8 μg/mL); and decreases in *PA* susceptibility to aztreonam or other antibiotics associated with changes in CFF Registry database clinical evaluations.
**Results:** 510 subjects enrolled (August to November, 2011). Mean age: 26 yrs (range 6 to 71); Subjects ⩾18 yrs: 377 (73.9%). Mean $FEV_1$ %predicted at enrollment: 60.4 (range 17–104). 278 subjects (54.5%) received ⩾1 AZLI course in the preceding 1 year; 130 (25.5%) received ⩾5 AZLI courses. Baseline sputum pathogens: *PA* (77%), *S. aureus* (MSSA 24%/MRSA 26%), *S. maltophilia* (11%), *Achromobacter* spp. (6%), *Burkholderia* spp. (2%), *Aspergillus* spp. (18%). Aztreonam $MIC_{50}/MIC_{90}$ for all *PA* isolates (612 *PA* strains/391 *PA*-positive samples) were 4 and 128 μg/mL; 38% of subjects had a *PA* isolate with an aztreonam MIC >8 μg/mL.
**Conclusions:** This observational cohort study provides opportunities to document changes in *PA* antibiotic resistance patterns and possible associations with clinical outcomes over a 5 yr period.
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## 62 A rapid biofilm antimicrobial susceptibility assay under 24 hours for cystic fibrosis patients with *Pseudomonas aeruginosa* lung infection

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**Objectives:** Minimum biofilm eradication concentration (MBEC) assay with peg-lid microtiter plate has been well developed for biofilm susceptibility test. But the method includes long-term assay of 4–6 days and biofilm formation difficulties in some mucoid *P. aeruginosa* strains. In this study a novel biofilm antimicrobial susceptibility assay is proposed as a development for guidance of antibiotic administration in cystic fibrosis (CF) patients.
**Methods:** *P. aeruginosa* PAO1 (non-mucoid), PDO300 (mucoid) and NH57388A (mucoid clinical strain from CF lung) were imbedded in alginate/agarose beads (50–100 μm in diameter). 0.1 ml of $5\times10^6$cfu/ml beads was added in 96-wells microtiter plate treated by 0.1 ml antibiotics for 4–8h in a minimal medium. Live/Dead-Kit was used to quickly detect the MBEC of antibiotics on the beads biofilm by fluorescent microscopy. After sonication, 0.1 ml samples were diluted and cultured on plates or added in LB media overnight to confirm the MBEC data from fluorescent microscopy. As a control, the peg-lid MBEC assay was performed in parallel. All experiments were repeated two times.
**Results:** MBEC of azithromycin, aztreonam, ceftazidime, ciprofloxacin, colistin, imipenem, meropenem, tobramycin was 4–1024 μg/ml by peg-lid microtiter plate method. Similar MBEC results were found in alginate/agarose beads biofilm with 8–1024 μg/ml. The results attained by Live/Dead-Kit agreed with the CFU-results. Time spent on peg-lid biofilm susceptibility assay was 5 days, but only 12–24h for alginate/agarose biofilm susceptibility assay.
**Conclusion:** Alginate/agarose biofilm antimicrobial susceptibility assay is a promising candidate method for clinical application.

## 63 Distribution of tobramycin and the impact on bacterial killing in a biofilm model

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Despite increased knowledge and research over the past decades, biofilm infections are still difficult to manage. We suggest considering biofilms as independent compartments with particular pharmacokinetics.
To examine our thesis the pharmacokinetics was related to the pharmacodynamics of tobramycin in an alginate-embedded biofilm model.
Seaweed alginate beads with *Pseudomonas aeruginosa* were prepared. The beads were placed in LB medium and grown at 37ºC. At day 1, 3, 5, or 7 bacteriology was determined for beads treated with tobramycin for 30 minutes in physiological treatment concentrations and non-treated beads. The beads were homogenized, serially diluted and the number of CFUs determined after overnight incubation. Tobramycin concentrations in the solution of homogenized beads were measured (Architect). Finally, beads were studied using a confocal laser scanning microscope (Zeiss Zen 2010). Live cells were visualized by Syto9 and dead cells by propidium iodidee staining.
Tobramycin concentrations were stable throughout our experiment (range 30–42 mg/l, MIC = 1.0 mg/l). CFU per bead were lower in the tobramycin treated as compared to the non treated group ($p < 0.016$). The quantitative and the relative bacterial killing were reduced from day 1 to day 7. Through the study period increasing sizes as well as more superficial positioning of the micro-colonies within the beads was demonstrated.
The present study, resembling physiological pharmacokinetics of tobramycin demonstrates a good distribution of tobramycin in an in vitro biofilm model. However, the effect was reduced as biofilms matured. The present model may add knowledge to optimize treatment of biofilm infections.